3. Materials and methods

3.1 Materials

Milk samples from bulk tank milk were collected from farm of National Pingtung University of Science and Technology and a local dairy farm located in Wandan Township, Pingtung, Taiwan. Milk samples from individual dairy cows were collected from farm of National Pingtung University of Science and Technology.

3.2 Methods

Two different experiments were performed in this study. In experiment I, milk samples from bulk tank in dairy farms were allotted into 3 groups according to total SCC, including group 1 with SCC below 200×10^3/mL, group 2 with SCC ranged from 200×10^3 to 500×10^3/mL, and group 3 with SCC ranged from 500×10^3 to 750×10^3/mL respectively (Figure 3.1). The grouping of Bulk milk somatic cell count according to Canadian Dairy Herd Improvement. In experiment II, milk samples from individual cows were collected from the healthy quarter (below 400×10^3/mL) and infected quarter of the same cow for comparing influence of mastitis on milk quality (Figure 3.2).

All milk samples were analyzed for Milk composition (fat, protein and lactose), pH, titratable acidity, ionic calcium, ethanol stabilty and total bacterial count.

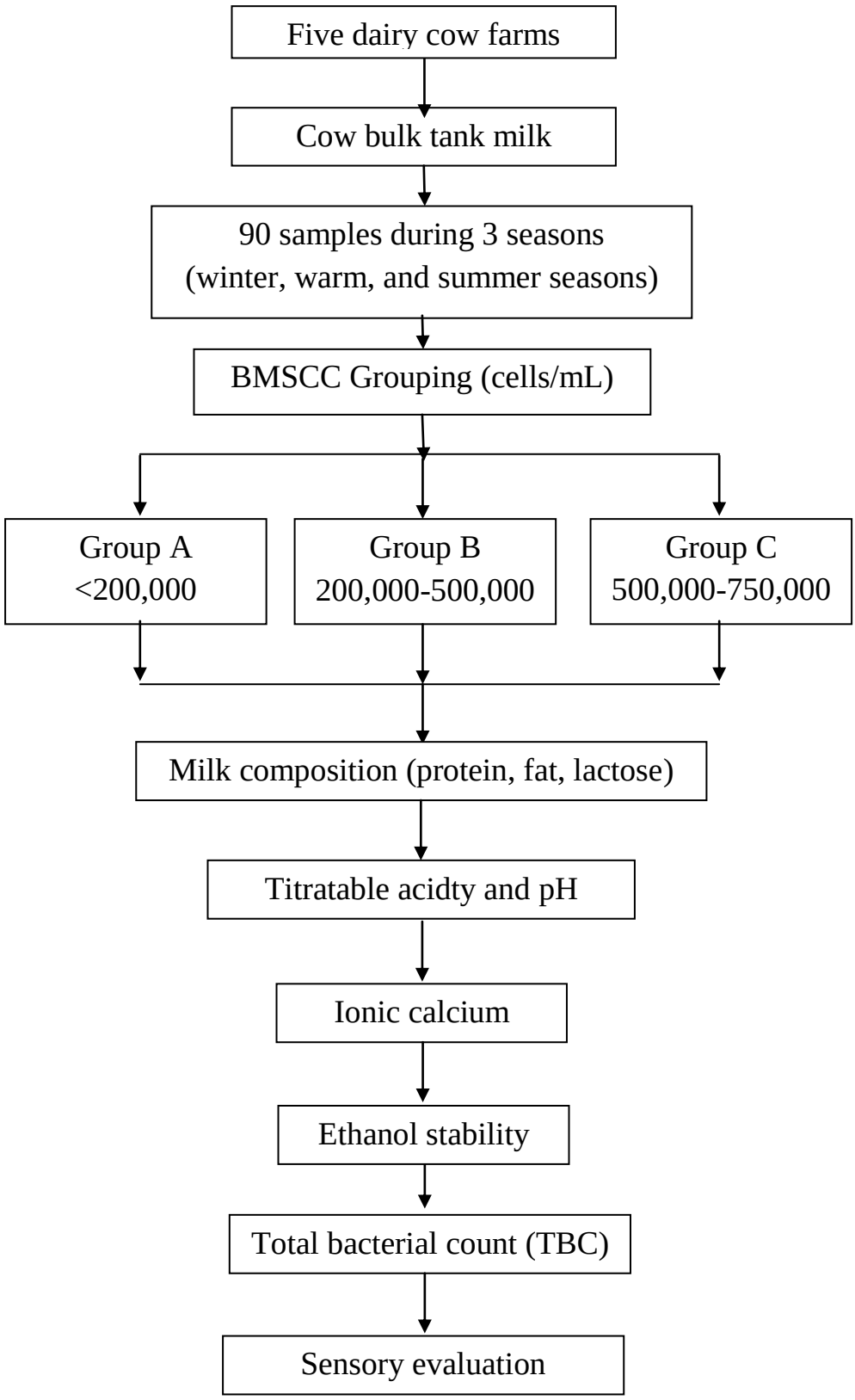

Figure 3.1 Procedure of experiment I, grouping of BMSCC according to Canadian Dairy Herd Improvement.

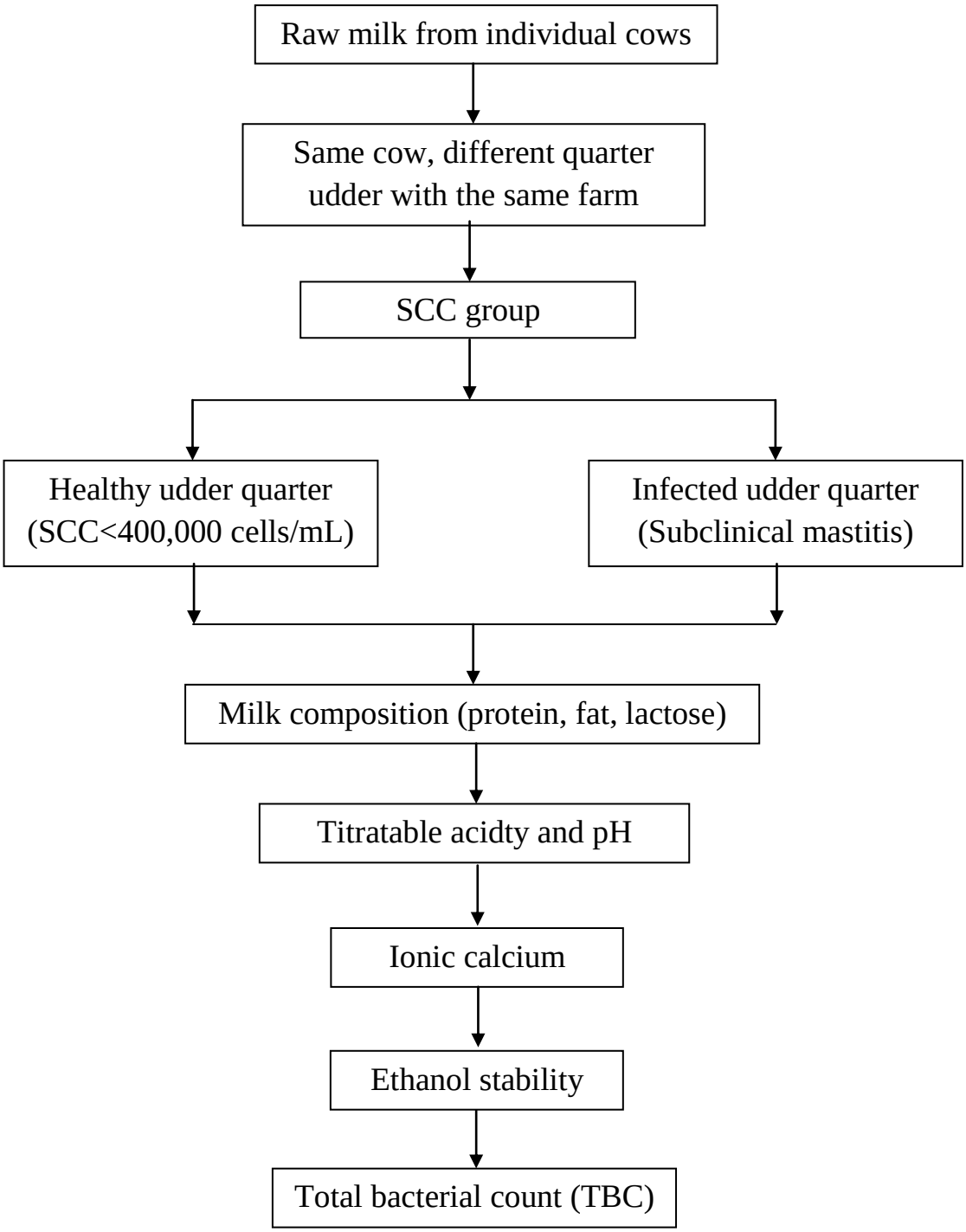

Figure 3.2 Procedure of experiment II, grouping of SCC according to Indonesia National Standard (SNI 3141.1:2011).

3.2.1 Somatic cell count

Somatic cell counts in milk are measured using a Nucleo counter (SCC-100, Denmark) (Figure 3.3). Raw milk 0.5 mL and 0.5 mL buffer (reagent C) (Chemometec company, Denmark) were pippet into 1.5 mL centrifuge tube and mixed for 10 second while avoiding bubbles. The mixture was drawn in a cassette. The cassette was put into the counter to take readings.

Figure 3.3 Nucleo counter SCC-100, Denmark.

3.2.2 Milk composition

The milk compositions (fat, protein, and lactose) were analyzed by using Milkoscope (Scope Electric ®, Expert-2059, Germany) (Figure 3.4), according to the manufacturer's instructions. Milk samples were mixed gently 6 times to avoid any air enclosure in the milk. Then, 50 mL milk samples were taken in the sample tube and put in the sample-holder one at a time with the analyzer in the recess position. Then when the starting button was activated, the analyzer draw the milk, makes the measurements, and returns the milk in the sample-tube and the digital indicator shows the specified results.

Figure 3.4 Milkoscope Electric ®, Expert-2059, Germany.

3.2.3 Titratable Acidity and pH

The pH meter (Suntex pH/ION meter SP-2500) (Figure 3.5) used in this study was calibrated against standart buffer solutions at pH 4.0 and 7.0. Titratable acidity (TA) as percentage of lactic acid was used to measure the total acidity. TA was measured by titrating 8.8 mL of sample and 9 mL of distilled water with 0.1 N sodium hydroxide (NaOH) using 2 drops phenolphthalein solution in 95% ethanol as the indicator.

Figure 3.5 Suntex pH/ION meter SP-2500.

3.2.4 Total bacterial count

Plate count agar (PCA) was used as medium for total bacterial counts. The medium was prepared by suspending 11.75 g in 500 mL distilled water. It was dissolved and autoclve at 121°C for 20 minutes. Nine mL of sodium chloride was poured into tubes and autoclaved. One mL sample was pipetted to a test tube (10^{-1} sample dilution) containing 9 mL of 0.9% sterilized sodium chloride solution. The test tube was then vortexed for a few seconds to obtain uniform mixture. 1 mL of liquid sample from the first test tube (10^{-1} sample dilution) was transferred to a second test tube (10^{-2} sample dilution). This procedure was repeated until 10^{-5} sample dilution. One mililiter of sample from tubes 10^{-2} to 10^{-5} were pippeted to petri dishes and 15 mL of sterilized PCA medium solution was then poured into each petri plate. The plates were ready to incubate (37 °C for 48 hours) when the agar solidified. The total numbers of bacterial count were calculated using the colony forming units (CFU) of plates containing 30-300 colonies after 48 hours of incubation.

3.2.5 Ethanol stability

Ethanol stability was measured by mixing equal volumes (1 mL) of milk sample and ethanol solution (water/ethanol ranging from 10 to 98%, v/v). For routine testing, 1 mL milk is mixed with 1 mL of ethanol solution. If the tested milk is of good quality, there will be no coagulation, clotting or precipitation. Presence of flakes or clots indicates poor quality milk.

3.2.6 Ionic calcium concentration

Ionic calcium was measured using the Suntex pH/ION SP-2500 analyser (Figure 3.5). The calibration solutions were calcium standart solutions in imizadole buffer at calcium consentrations in the ranges from 1.00 mM to 10.0 mM (Lin *et al.*, 2006). These were prepared by mixing a stock solution and dilution solution to give aqueous $CaCl_2$/KCl solutions, in an imidazole buffer at pH 6.7. The calcium stock solution was prepared by converting 2.0018g calcium carbonate ($CaCO_3$), which was

dried in an oven at 100 °C overnight, to calcium chloride with 5 M HCl into a 1000 mL flask and diluted with ultra pute water (MiliQ). Then, 1.361g imidazole and 0.5591g of KCl were added. The dilution solution was prepared by adding 1.3616g imidazole and 5.032g KCl into 1000 mL flask and diluted with deionized water. The pH value was adjusted to 6.70 by 1 M HCl or 1 M NaOH solution in both stock and dilution solutions before finally making up to the required volumes with deionized water. Different concentrations of ionic calcium were obtained mixing the different solution (e.g. in order to obtain a volume of 20 mL containing 1 mM of ionic calcium, 1 mL of stock solution was mixed with mixed19 mL of dilution solution) (Table 3.1).

Table 3.1 Ionic calcium standart solutions

Stock solution (mL)	Dilution solution (mL)	Volume (mL)	Final concentration (mM)
1.00	19.00	20	1.00
1.50	18.50	20	1.50
2.00	18.00	20	2.00
2.50	17.50	20	2.50
3.00	17.00	20	3.00
3.50	16.50	20	3.50
4.00	16.00	20	4.00
4.50	15.50	20	4.50
5.00	15.00	20	5.00

The ionic calcium measured of raw milk samples was carred out at room temperature. The potential difference value (mV) was measured by the immersion of the calcium ion selective electode for 1 minute in both the standart solution and the raw milk samples. The ionic calcium concentration (mM) was calculated based on standart curve showed in Figure 3.6.

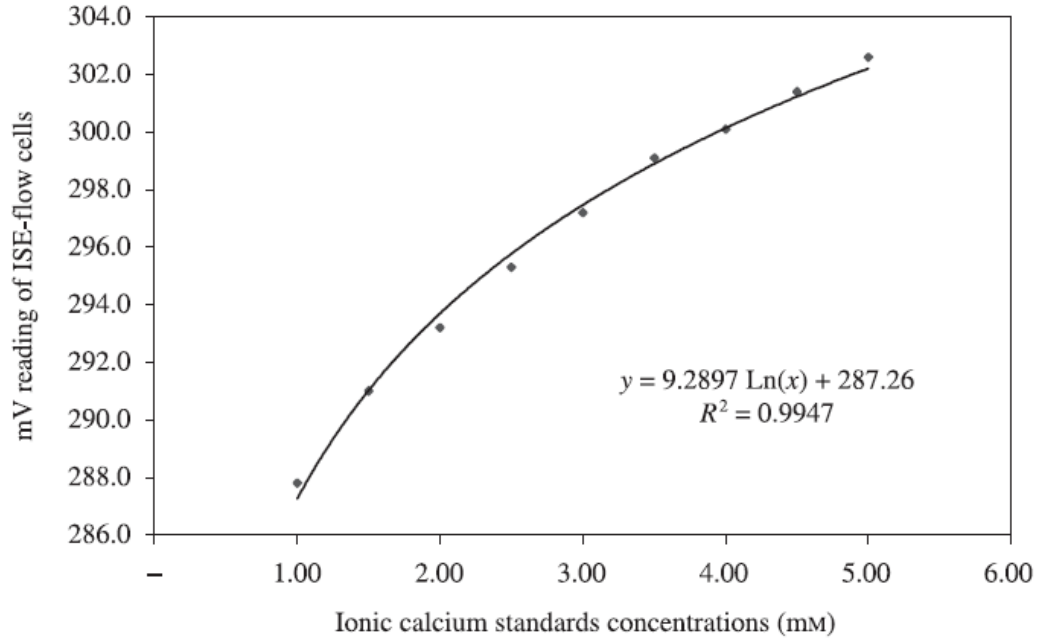

Figure 3.6 Ionic calcium standart curve.

3.2.7 Sensory evaluation

Students from National Pingtung University of Science and Technology (20-24 years old) were trained for sensory evaluation. To determine the effect of bulk milk somatic cell count on pasteurized milk descriptive test was performed with 30 trained panelists students. In the sensory test, 5 points hedonic scale was used, where the panelist scored from 1 to 5 according to their preference for the different levels of bulk milk somatic cell count (1=none, 2=very weak, 3=moderate, 4=strong, and 5=very strong). In descriptive test, sensory attributes such as aroma, rancidity, bitterness, and astringency were evaluated.

3.3 Characteristic of individual dairy cows

Variant of parity and stage of lactation in this study were list in Table 3.2.

Table 3.2 Variant of parity and stage of lactation in this study.

No. of cow	Parity	Lactation (days)
323	2	122
441	1	267
331	2	110
351	2	156
453	1	240
442	1	203
381	2	247
281	2	242
461	1	125
412	1	158

3.4 Characteristic of seasons environment

Seasonal variations in raw milk were categorised into four groups as shown in Table 3.3.

Table 3.3 Variant of temperature and relative humidity in different season

Seasons	Months	Temperature (°C)	Humidity (%)
Winter	December, January and February	15.2 – 22.4	62 – 76
Spring	March, April and May	20.4 – 28.1	69 – 80
Summer	June, July and August	27.4 – 32.2	78 – 95

(Central Weather Bureau of Taiwan, 2016-2017)

3.5 Statistical Analysis

Statistical Analysis System (version 9.3, SAS) was used to perform data analysis using the general linear model (GLM). Duncan multiple range test was used for comparison of mean of treatments. Correlation coefficient was also established to investigate relationships between parameters.